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Several studies have suggested an association between low penetrant alleles and breast cancer risk. Although the contribution of low penetrant alleles to the individual risk is relatively small, they can contribute to a large proportion of breast cancer cases in the population. In this study we took the candidate gene approach to study the association of 21 different genetic polymorphisms with breast cancer risk in a population-based sample using a high-throughput genotyping technology. To date, we have established and validated the genotyping methods. We have completed genotyping 398 cases and 372 population controls for 21 SNPs from several cancer-related molecular pathways. Initial statistical analysis of the cases and controls has shown that XPD cod751 polymorphism is significantly associated with breast cancer risk. Further analysis of the cases has shown that SNPs of ER, XPD, COMT and p27 genes were significantly associated with breast cancer risk in breast cancer cases with at least a first-degree relative of breast cancer. Statistical analysis to investigate the gene-gene and gene-environmental interactions of the SNPs is currently ongoing. This project has the potential to identify breast cancer susceptibility variants in the context of interaction with other genetic or epidemiological risk factors.

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OVERVIEW

It has long been hypothesized that genetic variation is responsible for observed differences in cancer risk and susceptibility amongst the human population. Mutant alleles of dominant highly penetrant breast cancer genes, including BRCA1 and BRCA2 (1-3), do not occur frequently, and hence account for only a small proportion of breast cancer cases. On the other hand, several studies have suggested an association between low penetrant alleles and breast cancer risk. Although the contribution of low penetrant alleles to the individual breast cancer risk is relatively small, they can contribute to a large proportion of breast cancer cases in the population because the risk-conferring alleles of these genes are common.

Identification and cloning of low penetrant alleles that increase the risk of breast cancer is challenging because the association methods for such studies require large populations to achieve meaningful statistical analysis and very dense genetic maps to facilitate genome-wide genotyping (4,5). At present, the candidate gene approach remains the most logical and practical strategy to identify these risk enhancing, low penetrant variants or single nucleotide polymorphisms (SNPs). Until now, a major obstacle with investigating the risk associated with multiple candidate genes has been a lack of technology for large-scale genotyping of large populations. Consequently, many studies have focused efforts on only 1 or 2 genetic polymorphisms, and even in these cases the analysis was only limited to relatively small sample sizes. In the context of the ideas program, we exploited the high throughput power of SNP genotyping technologies and a well defined, representative population-based sample containing a large number of subjects. We have selected genetic polymorphisms in genes involved in different aspects of carcinogenesis (7-41). For example, cell cycle regulatory genes such as CDK-inhibitors, and cyclins; carcinogen metabolizing enzymes such as CYPs, GSTs and NATs; immune system genes such as interluekins and TNF; and genes involved in other pathways involved in cancer (e.g. p53, PTEN, XPD-DNA repair gene). We have access to the Ontario Familial Breast Cancer Registry (OFBCR), which is the largest population based breast cancer registry in Canada.

The main objective of the proposed work is to identify low penetrant, yet commonly occurring, genetic polymorphisms, which contribute to the risk of developing breast cancer. Furthermore, this approach has the potential to identify novel genetic factors associated with breast cancer risk, which may result in the development of innovative therapies, and a fuller understanding of genetic variation in response to therapy. This will lead to a more complex analysis of gene-gene and gene-environment interactions than is currently possible. Advances in disease etiology will significantly expand our abilities to design strategies for the prevention of breast cancer development and progression.

STATEMENT OF WORK

Task 1: Characterization of polymorphic alleles by SSCP, Months 1-8

a. Design of SSCP primers for 32 sites
b. Screen by SSCP analysis for all possible alleles at each locus
c. Sequence the SSCP patterns (appr 3 per loci) and identify the all possible genotypes

Task 2: Designing of oligonucleotides and sample microarrays, Months 4-8

a. Design different sets of oligonucleotides (perfect matches and mismatches)
b. Customize sample chips for quality control of hybridizations

Task 3: Optimization of the hybridizations using PCR probes, Months 8-16

a. Prepare PCR probes using control specimens
b. Optimize the hybridization conditions
c. Evaluate the accuracy of detection for every polymorphic site using a probes with different allelic combinations for each polymorphism
d. Redesign oligonucleotides and chips in order to increase the quality and accuracy of detection

Task 4: Genotyping of 900 specimens for 32 polymorphisms, Months 16-32

a. Production of microarray chips
b. Preperation of flourescent labelled PCR probes for each patient
c. Hybridization of chips at optimized conditions
d. Reading and analysis of the chip signals
e. Quality control experiments at different intervals using the control specimens to ensure the reproducibility of results

Task 5: Data and statistical analysis, Months 32-36

a. Repeat and conformation experiments
b. Complete the reading of every slide and prepare the data for statistical analysis
c. Univariate analysis of the data
d. Exploratory multivariate analysis of the data

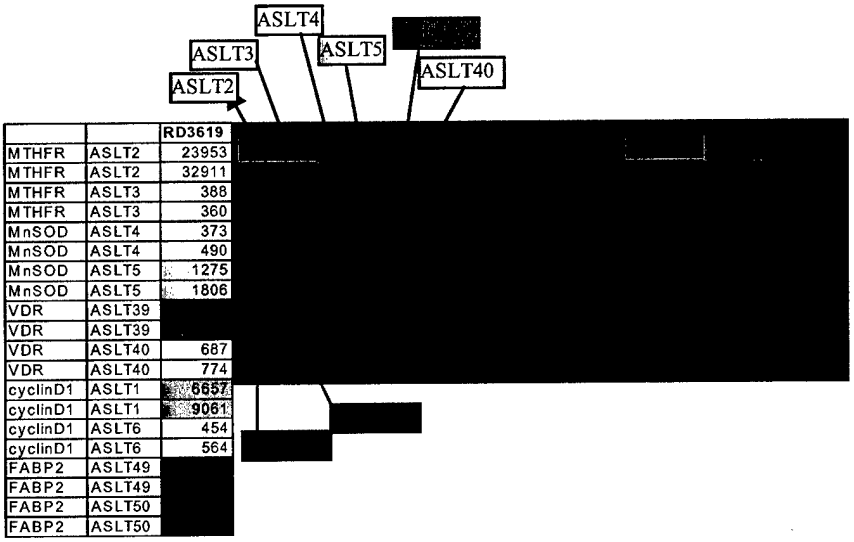
BODY

A. Establishment of Genotyping Methods

A1. SNParrays

Support oligonucleotides are designed to be printed on SNParrays and bind allele specific probes. Each support-oligonucleotide contains an anti-TAG sequence, which is complementary to the TAG-sequences on each allele-specific oligonucleotide. Each anti-TAG-sequence is also attached to a 15mer poly (T)-tail, which is designed to increase the efficiency of support-oligonucleotides to bind to the glass surface during printing. The SNParrays are printed on poly-L-lysine coated slides, according to the design given below (Figure 1), by the microarray facility of Samuel Lunenfeld Research Institute (SLRI) of Mount Sinai Hospital (MSH) in Toronto. Each support oligonucleotide is printed in duplicate for validation purposes. The spots on the slides are rehydrated and snap-dried after printing process, and are fixed in a UV cross-linker at 600mJ. Unbound oligonucleotides and excess salt was washed off the slides.

PCR reactions for probe preparation were performed in a total volume of 10□l, in the presence of 10□M of each cold dNTP, a range of 2-4mM $MgCl_2$, 5pmol of each allele specific primer, 10pmol of the common reverse primer, 5□M of fluorescently (cy5) labeled dCTP, 0.25U of Platinum Taq polymerase, and 10ng genomic DNA. Different annealing temperatures were used (ranging from 55-65^{0}C), depending on the melting temperatures of the PCR primers used. For the quality control purposes, previously known homozygote and heterozygote templates are used to prepare the probes for each SNP. Each probe is hybridized to duplicate arrays to ensure that the genotypes were detected correctly. Five microliters of the PCR reactions (probes) are pooled, and 15□l of this mix was mixed with 5 □l of a hybridization mixture (1.33xSSC, 0.067% SDS, 0.033mg/ml of salmon sperm DNA). This mixture was hybridized to the SNParray under a coverslip for 3 hrs at 50^{0}C. The slides were then washed, dried in a centrifuge, scanned in a GenePix 4000B slide scanner (Axon) and analyzed with a Genepix Pro 4.0 analysis software (Figure 1). The signal intensities detected by this software converted to genotypes by another software specifically written by SLRI microarray facility.



		RD3619
MTHFR	ASLT2	23953
MTHFR	ASLT2	32911
MTHFR	ASLT3	388
MTHFR	ASLT3	360
MnSOD	ASLT4	373
MnSOD	ASLT4	490
MnSOD	ASLT5	1275
MnSOD	ASLT5	1806
VDR	ASLT39	
VDR	ASLT39	
VDR	ASLT40	687
VDR	ASLT40	774
cyclinD1	ASLT1	6657
cyclinD1	ASLT1	9061
cyclinD1	ASLT6	454
cyclinD1	ASLT6	564
FABP2	ASLT49	
FABP2	ASLT49	
FABP2	ASLT50	
FABP2	ASLT50	

Gene	Genotype
MTHFR	Nt677 C/C
SOD	Nt-9 T/T
VDR	Cdn1 T/T
CyclinD1	Cdn241 A/A
FABP2	Cdn54 G/A

Figure 1: Output of a SNParray hybridization. The signal intensities (left table) were obtained after processing of the raw data taking into consideration the background intensities. Genotypes (right table) were obtained on the basis of signal ratios.

A2. TaqMan, a 5' Nuclease Assay

This method uses the Perkin-Elmer (PE) Applied Biosystems Sequence Detection 7900 HT System. This PCR based detection method uses allele-specific fluorescent probes, with a different label for each allele, to discriminate between alleles. Probes anneal in a sequence-specific manner between the PCR primers, and in the course of the PCR the 5'-nuclease activity of the *Taq* polymerase releases the reporter dye of bound probes only, emitting an allele-specific fluorescence. The reporter fluorescent signal of probes is subdued by a quencher molecule in the intact probe, and does not release a signal. This methodology has the advantages of avoiding the use of restriction digests, hybridizations or electrophoresis thereby avoiding many sources of error and allowing high-throughput genotyping.

Oligonucleotide primer and the dual labelled allele specific probe sequences were designed using PrimerExpress version 2.0 (PE Biosystems). Amplification reactions were performed in 96 well plates (Figure 2). Each plate contained four control DNAs for each possible genotype. Genomic DNA (5ng) was amplified in a total volume of 10 □l in the presence of 100 □M of each of the dNTPs, 3 pmoles of each of the appropriate primers, 2 pmoles of each of the corresponding dual labeled probes, and 0.025 Unit of Platinum Taq DNA Polymerase (InVitrogen). The Mg concentrations varied between 2 and 4 mM depending on the SNP studied. A home-made PCR buffer was used in 1X concentration in the reactions. PCR cycling conditions consisted of 40 cycles of 94^0C for 15 sec, X^0C for 15 sec and 72^0C for 15 sec, annealing temperature also varied between 58^0C and 64^0C, depending on the Tm of specific probes and primers. The reactions were analyzed by ABI PRISM 7900HT Sequence Detection System (version 2.0) (Figure 3).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Case1	Case9	Case16	Case24	Case32	Case39	Case47	Case55	Case62	Control 1	Control 8	Homozygote 2
B	Case2	Case10	Case17	Case25	Case33	Case40	Case48	Case56	Case63	Control 2	Control 9	Homozygote 2
C	Case3	ND	Case18	Case26	Case34	Case41	Case49	Case57	Case64	Control 3	Control 10	Homozygote 2
D	Case4	Case11	Case19	Case27	Case35	Case42	Case50	Case58	Case65	Control 4	Control 11	Homozygote 2
E	Case5	Case12	Case20	Case28	ND	Case43	Case51	Case59	Case66	Control 5		
F	Case6	Case13	Case21	Case29	Case36	Case44	Case52	ND	Case67	Control 6		
G	Case7	Case14	Case22	Case30	Case37	Case45	Case53	Case60	Case68	Control 7		
H	Case8	Case15	Case23	Case31	Case38	Case46	Case54	Case61	Case69	ND		

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control 1	Control 9	Control 16	Control 24	Control 32	Control 39	Control 47	Control 55	Control 62	Case 1	Case 8	Homozygote 2
B	Control 2	Control 10	Control 17	Control 25	Control 33	Control 40	Control 48	Control 56	Control 63	Case 2	Case 9	Homozygote 2
C	Control 3	ND	Control 18	Control 26	Control 34	Control 41	Control 49	Control 57	Control 64	Case 3	Case 10	Homozygote 2
D	Control 4	Control 11	Control 19	Control 27	Control 35	Control 42	Control 50	Control 58	Control 65	Case 4	Case 11	Homozygote 2
E	Control 5	Control 12	Control 20	Control 28	ND	Control 43	Control 51	Control 59	Control 66	Case 5		
F	Control 6	Control 13	Control 21	Control 29	Control 36	Control 44	Control 52	ND	Control 67	Case 6		
G	Control 7	Control 14	Control 22	Control 30	Control 37	Control 45	Control 53	Control 60	Control 68	Case 7		
H	Control 8	Control 15	Control 23	Control 31	Control 38	Control 46	Control 54	Control 61	Control 69	ND		

Figure 2: Design of case and control DNAs on the 96-well microplate.

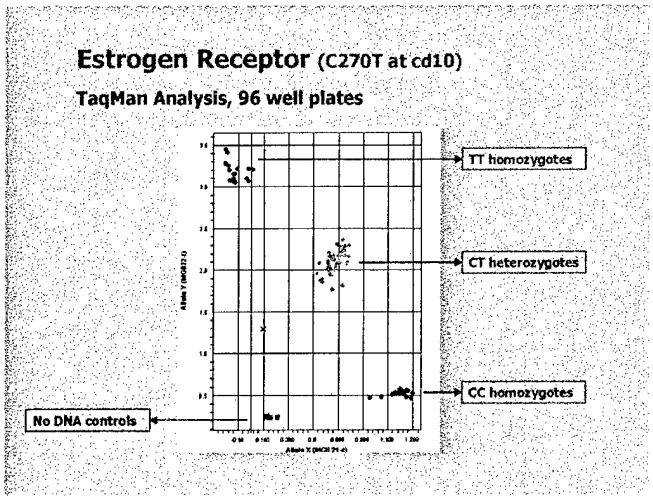


Figure 3: Output from Taqman assay. Genotypes (CC, TT and CT) are accumulated at different coordinates due to their signal intensity. This is done automatically by TaqMan software.

B. Validation of Genotyping Methods

In order to assess the specificity for SNP genotyping, we have carried out a validation study where a panel of 150 breast cancer cases and population controls were screened with both SNParray and Taqman methods for all the 21 SNPs in the study. Whereas in over 50% SNPs the results from both methods was concordant, Approximately 20% have shown highly discordant results. The remaining SNPs were more comparable to each other. We have repeated a fraction of the discordant results using the two methods complemented by direct sequencing. The differences in results between two methods arouse from poor signal intensity and high background content. Our validation study has shown that with our current setup, Taqman method has provided more reproducible and reliable genotyping results compared to SNParrays. Within the task and the budget of this proposal we have established an high-throughput SNP genotyping platform and carried out extensive method validation.

C. Genotyping of Cases and Controls

In this study we have genotyped 398 breast cancer cases and 372 population controls using the Taqman method. Approximately 25% of all cases and controls were genotyped by using both Taqman and SNParrays. Each 96-well microplate included multiple numbers of cell line DNA specimens representing all possible genotypes of each SNP screened. Furthermore, each micro-plate was designed to contain 10% repeat sample. The frequency distribution of SNPs screened successfully is provided according to the molecular pathways they represent (Table 1).

Table 1: Frequency table for all SNPs

		CASES			CONTROLS					
SNP	Genotype	Observed Number	Observed Frequency	Allele Frequency	Observed Number	Observed Frequency	Expected Number	Expected Frequency	Allele Frequency	X^2 test
Immune System Genes										
IL1B-Phe105Phe	CC	231	0.580	0.753	226	0.608	224	0.601	0.776	0.023
	CT	137	0.344		125	0.336	130	0.348		0.157
	TT	30	0.075	0.247	21	0.056	19	0.050	0.224	0.272
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.452***
G-CSF-Leu185Leu	AA	146	0.367	0.611	136	0.366	143	0.384	0.620	0.326
	AG	194	0.487		189	0.508	175	0.471		1.062
	GG	58	0.146	0.389	47	0.126	54	0.145	0.380	0.865
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***2.253***
IL13-Arg130Gln	AA	13	0.033	0.193	15	0.040	12	0.033	0.183	0.531
	AG	128	0.322		106	0.285	111	0.299		0.238
	GG	257	0.646	0.807	251	0.675	248	0.668	0.817	0.027
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.796***
TNFA-G-308A	AA	11	0.028	0.165	11	0.030	12	0.031	0.176	0.025
	AG	109	0.274		109	0.293	108	0.290		0.011
	GG	278	0.698	0.835	252	0.677	253	0.679	0.824	0.001
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.036***
IL1A-Ala114Ser	GG	198	0.497	0.696	193	0.519	196	0.527	0.726	0.045
	GT	158	0.397		154	0.414	148	0.398		0.238
	TT	42	0.106	0.304	25	0.067	28	0.075	0.274	0.315
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.598***
IL10-G-1082A	AA	105	0.266	0.513	104	0.280	102	0.275	0.524	0.031
	AG	194	0.492		182	0.489	186	0.499		0.068
	GG	95	0.241	0.487	86	0.231	84	0.226	0.476	0.038
	TOTAL	*394*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.137***
Cell Cycle Genes										
CyclinD1-Pro241Pro	AA	99	0.249	0.495	85	0.228	79	0.213	0.461	0.445
	AG	196	0.492		173	0.465	185	0.497		0.762
	GG	103	0.259	0.505	114	0.306	108	0.290	0.539	0.326
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***1.534***
p21-Ser31Arg	AA	2	0.005	0.080	2	0.005	2	0.004	0.066	0.093
	AC	60	0.151		45	0.121	46	0.123		0.013
	CC	336	0.844	0.920	325	0.874	325	0.873	0.934	0.000
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.106***
p27-Val109Gly	GG	16	0.040	0.190	15	0.040	14	0.037	0.194	0.081
	GT	119	0.299		114	0.306	116	0.312		0.039
	TT	263	0.661	0.810	243	0.653	242	0.650	0.806	0.005
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.125***
GADD45-IVS3+168	CC	32	0.080	0.288	36	0.097	36	0.097	0.312	0.001
	CT	165	0.415		160	0.430	160	0.429		0.001
	TT	201	0.505	0.712	176	0.473	176	0.474	0.688	0.000
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.002***
PTEN-IVS4+109	del/del	179	0.450	0.667	186	0.500	182	0.488	0.699	0.101
	ins/del	173	0.435		148	0.398	157	0.421		0.468
	ins/ins	46	0.116	0.333	38	0.102	34	0.091	0.301	0.543
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***1.112***

Table 1 continued: Frequency table for all SNPs

		CASES			CONTROLS					
SNP	Genotype	Observed Number	Observed Frequency	Allele Frequency	Observed Number	Observed Frequency	Expected Number	Expected Frequency	Allele Frequency	X^2 test
Estrogen and Carcinogen Metabolism Genes										
ESR1-Ser10Ser	CC	95	0.239	0.490	87	0.234	88	0.235	0.485	0.004
	CT	200	0.503		187	0.503	186	0.500		0.007
	TT	103	0.259	0.510	98	0.263	99	0.265	0.515	0.003
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.015***
ESR1-Pro325Pro	CC	235	0.590	0.764	217	0.583	215	0.577	0.759	0.026
	CG	138	0.347		131	0.352	136	0.365		0.179
	GG	25	0.063	0.236	24	0.065	22	0.058	0.241	0.283
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.488***
CYP17-CACbox	CC	64	0.161	0.393	47	0.126	44	0.117	0.343	0.249
	CT	185	0.465		161	0.433	168	0.451		0.260
	TT	149	0.374	0.607	164	0.441	161	0.432	0.657	0.068
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.577***
COMT-Met158Val	AA	91	0.229	0.492	105	0.282	103	0.278	0.527	0.029
	AG	210	0.528		182	0.489	185	0.499		0.065
	GG	97	0.244	0.508	85	0.228	83	0.224	0.473	0.036
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.130***
GSTM3-IVS6+20	del/del	11	0.028	0.160	11	0.030	11	0.028	0.168	0.024
	ins/del	105	0.264		103	0.277	104	0.280		0.010
	ins/ins	281	0.708	0.840	258	0.694	258	0.692	0.832	0.001
	TOTAL	*397*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.034***
GSTP1-Ile105Val	AA	178	0.448	0.666	177	0.476	176	0.474	0.688	0.004
	AG	173	0.436		158	0.425	160	0.429		0.017
	GG	46	0.116	0.334	37	0.099	36	0.097	0.312	0.019
	TOTAL	*397*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.040***
DNA Repair and Other Cancer Related Genes										
XPD-Lys751Gln	AA	155	0.389	0.623	172	0.462	171	0.459	0.677	0.010
	AC	186	0.467		160	0.430	163	0.437		0.041
	CC	57	0.143	0.377	40	0.108	39	0.104	0.323	0.043
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.094***
MTHFR-Ala222Val	CC	153	0.384	0.619	152	0.409	154	0.415	0.644	0.031
	CT	187	0.470		175	0.470	171	0.459		0.113
	TT	58	0.146	0.381	45	0.121	47	0.127	0.356	0.102
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.246***
BARD1-Pro24Ser	CC	171	0.430	0.661	140	0.376	145	0.391	0.625	0.194
	CT	184	0.462		185	0.497	174	0.469		0.647
	TT	43	0.108	0.339	47	0.126	52	0.141	0.375	0.540
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***1.381***
MMP1-(-1607) insG	del/del	107	0.269	0.525	95	0.255	94	0.253	0.503	0.011
	ins/del	204	0.513		184	0.495	186	0.500		0.021
	ins/ins	87	0.219	0.475	93	0.250	92	0.247	0.497	0.011
	TOTAL	*398*	*1.000*	*1.000*	*372*	*1.000*			*1.000*	***0.043***

D. Statistical Analysis

We have started the statistical analysis of the data. For each SNP, we tested for an association under dominant and recessive models, although the power for the recessive model is low for rarer alleles. Currently, logistic regression has been applied to the analysis of case control data for each SNP (whole analysis) under dominant and recessive models (Table 2). Among the genes studied DNA repair gene, XPD Lys751Gln SNP has shown statistically significant association with breast cancer risk (bolded in Table 2). On the other hand multiple other SNPs studied (IL-1a, cyp17, COMMT, PTEN, Cyclin D1, BARD) has shown borderline significance.

We have also carried out the statistical analysis on the basis of presence or absence of family history of breast cancer (Table 3). Interestingly we have shown that ER, XPD, COMMT, and P27 genes have shown significant associations with breast cancer risk in cases with first degree relatives with breast cancer. Non of the SNPs has shown association in cases where there was no first degree relative with breast cancer (results are not shown).

Table 2: Statistical Analysis of Cases and Controls

Immune System Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
IL1B-Phe105Phe			
CC	231 (58.04)	226 (60.75)	1
CT	137 (34.42)	125 (33.6)	1.072 (0.791-1.453)
TT	30 (7.54)	21 (5.65)	1.398 (0.777-2.514)
CT or TT	167 (41.96)	146 (39.25)	1.119 (0.839-1.492)
G-CSF-Leu185Leu			
AA	146 (36.68)	136 (36.56)	1
AG	194 (48.74)	189 (50.81)	0.956 (0.703-1.301)
GG	58 (14.57)	47 (12.63)	1.15 (0.733-1.803)
AG or GG	252 (63.32)	236 (63.44)	0.995 (0.742-1.334)
IL13-Arg130Gln			
Arg/Arg	257 (64.57)	251 (67.47)	1
Arg/Gln	128 (32.16)	106 (28.49)	1.179 (0.864-1.609)
Gln/Gln	13 (3.27)	15 (4.03)	0.846 (0.395-1.815)
Arg/Gln or Gln/Gln	141 (35.43)	121 (32.53)	1.138 (0.844-1.534)
TNFA-G-308A			
GG	278 (69.85)	252 (67.74)	1
AG	109 (27.39)	109 (29.3)	0.906 (0.661-1.243)
AA	11 (2.76)	11 (2.96)	0.906 (0.386-2.127)
AG or AA	120 (30.15)	120 (32.26)	0.906 (0.668-1.230)
IL1A-Ala114Ser			
Ala/Ala	198 (49.75)	193 (51.88)	1
Ala/Ser	158 (39.7)	154 (41.4)	1 (0.743-1.347)
Ser/Ser	42 (10.55)	25 (6.72)	1.638 (0.961-2.791)
Ala/Ser or Ser/Ser	200 (50.25)	179 (48.12)	1.089 (0.821-1.445)
IL10-G-1082A			
AA	105 (26.65)	104 (27.96)	1
AG	194 (49.24)	182 (48.92)	1.056 (0.753-1.481)
GG	95 (24.11)	86 (23.12)	1.094 (0.735-1.629)
AG or GG	289 (73.35)	268 (72.04)	1.068 (0.777-1.468)
Cell Cycle Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
CyclinD1-Pro241Pro			
GG	103 (25.88)	114 (30.65)	1
AG	196 (49.25)	173 (46.51)	1.254 (0.896-1.754)
AA	99 (24.87)	85 (22.85)	1.289 (0.869-1.911)
AG or AA	295 (74.12)	258 (69.35)	1.265 (0.924-1.733)
p21-Ser31Arg			
Ser/Ser	336 (84.42)	325 (87.37)	1
Ser/Arg	60 (15.08)	45 (12.1)	1.29 (0.851-1.954)
Arg/Arg	2 (0.5)	2 (0.54)	0.967 (0.135-6.907)

Ser/Arg or Arg/Arg	62 (15.58)	47 (12.63)	1.276 (0.848-1.920)
p27-Val109Gly			
Val/Val	263 (66.08)	243 (65.32)	1
Val/Gly	119 (29.9)	114 (30.65)	0.964 (0.707-1.316)
Gly/Gly	16 (4.02)	15 (4.03)	0.986 (0.477-2.036)
Val/Gly or Gly/Gly	135 (33.92)	129 (34.68)	0.967 (0.718-1.302)
GADD45-IVS3+168			
TT	201 (50.5)	176 (47.31)	1
CT	165 (41.46)	160 (43.01)	0.903 (0.671-1.215)
CC	32 (8.04)	36 (9.68)	0.778 (0.464-1.306)
CT or CC	197 (49.5)	196 (52.69)	0.88 (0.663-1.168)
PTEN-IVS4+109			
del/del	179 (44.97)	186 (50)	1
del/ins	173 (43.47)	148 (39.78)	1.215 (0.899-1.640)
ins/ins	46 (11.56)	38 (10.22)	1.258 (0.781-2.025)
ins/ins or del/ins	219 (55.03)	186 (50)	1.223 (0.922-1.624)
Estrogen and Carcinogen Metabolism Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
ER-Ser10Ser			
TT	103 (25.88)	98 (26.34)	1
CT	200 (50.25)	187 (50.27)	1.018 (0.724-1.431)
CC	95 (23.87)	87 (23.39)	1.039 (0.695-1.552)
CT or CC	295 (74.12)	274 (73.66)	1.024 (0.743-1.413)
ER-Pro325Pro			
CC	235 (59.05)	217 (58.33)	1
CG	138 (34.67)	131 (35.22)	0.973 (0.719-1.316)
GG	25 (6.28)	24 (6.45)	0.962 (0.533-1.735)
CG or GG	163 (40.95)	155 (41.67)	0.971 (0.729-1.294)
CYP17-CACbox			
TT	149 (37.44)	164 (44.09)	1
CT	185 (46.48)	161 (43.28)	1.265 (0.931-1.718)
CC	64 (16.08)	47 (12.63)	1.499 (0.968-2.320)
CT or CC	249 (62.56)	208 (55.91)	1.318 (0.988-1.758)
COMT-Met158Val			
Met/Met	91 (22.86)	105 (28.23)	1
Met/Val	210 (52.76)	182 (48.92)	1.331 (0.944-1.877)
Val/Val	97 (24.37)	85 (22.85)	1.317 (0.879-1.973)
Met/Val or Val/Val	307 (77.14)	267 (71.77)	1.327 (0.958-1.836)
GSTM3-IVS6+20			
ins/ins	281 (70.78)	258 (69.35)	1
ins/del	105 (26.45)	103 (27.69)	0.936 (0.680-1.289)
del/del	11 (2.77)	11 (2.96)	0.918 (0.391-2.154)
del/del or ins/del	116 (29.22)	114 (30.65)	0.934 (0.686-1.272)
GSTP1-Ile105Val			
AA	178 (44.84)	177 (47.58)	1
AG	173 (43.58)	158 (42.47)	1.089 (0.807-1.469)
GG	46 (11.59)	37 (9.95)	1.236 (0.765-1.998)
AG or GG	219 (55.16)	195 (52.42)	1.117 (0.841-1.483)
DNA Repair and Other Cancer Related Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
XPD-Lys751Gln			
Lys/Lys	155 (38.94)	172 (46.24)	1
Lys/Gln	186 (46.73)	160 (43.01)	1.29 (0.953-1.746)
Gln/Gln	57 (14.32)	40 (10.75)	1.581 (0.999-2.502)
Gln/Gln or Lys/Gln	243 (61.06)	200 (53.76)	**1.348 (1.012-1.796)**
MTHFR-Ala222Val			
Ala/Ala	153 (38.44)	152 (40.86)	1
Ala/Val	187 (46.98)	175 (47.04)	1.062 (0.783-1.440)
Val/Val	58 (14.57)	45 (12.1)	1.28 (0.817-2.007)
Ala/Val or Val/Val	245 (61.56)	220 (59.14)	1.106 (0.829-1.477)
BARD1-Pro24Ser			
Pro/Pro	171 (42.96)	140 (37.63)	1
Pro/Ser	184 (46.23)	185 (49.73)	0.814 (0.602-1.102)
Ser/Ser	43 (10.8)	47 (12.63)	0.749 (0.468-1.199)
Pro/Ser or Ser/Ser	227 (57.04)	232 (62.37)	0.801 (0.600-1.069)
MMP1-(-1607) insG			
del/del	107 (26.88)	95 (25.54)	1
del/ins	204 (51.26)	184 (49.46)	0.984 (0.700-1.384)
ins/ins	87 (21.86)	93 (25)	0.831 (0.555-1.242)
ins/ins or del/ins	291 (73.12)	277 (74.46)	0.933 (0.676-1.287)

Table 3: Analysis based on the family history of breast cancer

Immune System Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
IL1B-Phe105Phe			
CC	49 (61.25)	23 (65.71)	1
CT	26 (32.5)	10 (28.57)	1.22 (0.505-2.947)
TT	5 (6.25)	2 (5.71)	1.173 (0.212-6.508)
CT or TT	31 (38.75)	12 (34.29)	1.213 (0.529-2.782)
G-CSF-Leu185Leu			
AA	24 (30)	9 (25.71)	1
AG	43 (53.75)	23 (65.71)	0.701 (0.280-1.756)
GG	13 (16.25)	3 (8.57)	1.625 (0.373-7.072)
AG or GG	56 (70)	26 (74.29)	0.808 (0.330-1.979)
IL13-Arg130Gln			
Arg/Arg	50 (62.5)	25 (71.43)	1
Arg/Gln	26 (32.5)	9 (25.71)	1.444 (0.589-3.543)
Gln/Gln	4 (5)	1 (2.86)	2 (0.212-18.848)
Arg/Gln or Gln/Gln	30 (37.5)	10 (28.57)	1.5 (0.634-3.551)
TNFA-G-308A			
GG	57 (71.25)	21 (60)	1
AG	21 (26.25)	13 (37.14)	0.595 (0.253-1.397)
AA	2 (2.5)	1 (2.86)	0.737 (0.063-8.556)
AG or AA	23 (28.75)	14 (40)	0.605 (0.263-1.390)
IL1A-Ala114Ser			
Ala/Ala	44 (55)	22 (62.86)	1
Ala/Ser	28 (35)	10 (28.57)	1.4 (0.578-3.392)
Ser/Ser	8 (10)	3 (8.57)	1.333 (0.322-5.528)
Ala/Ser or Ser/Ser	36 (45)	13 (37.14)	1.385 (0.613-3.128)
IL10-G-1082A			
AA	19 (24.05)	11 (31.43)	1
AG	45 (56.96)	19 (54.29)	1.371 (0.549-3.427)
GG	15 (18.99)	5 (14.29)	1.737 (0.495-6.094)
AG or GG	60 (75.95)	24 (68.57)	1.447 (0.600-3.492)
Cell Cycle Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
CyclinD1-Pro241Pro			
GG	25 (31.25)	12 (34.29)	1
AG	38 (47.5)	13 (37.14)	1.403 (0.552-3.566)
AA	17 (21.25)	10 (28.57)	0.816 (0.288-2.311)
AG or AA	55 (68.75)	23 (65.71)	1.148 (0.494-2.667)
p21-Ser31Arg			
Ser/Ser	67(83.75)	31 (88.57)	1
Ser/Arg	12(15)	4 (11.43)	1.388 (0.414-4.650)
Arg/Arg	1(1.25)	0 (0)	na (na)
Ser/Arg or Arg/Arg	13 (16.25)	4 (11.43)	1.503 (0.453-4.985)
p27-Val109Gly			
Val/Val	44 (55)	26 (74.29)	1
Val/Gly	34 (42.5)	8 (22.86)	**2.511 (1.011-6.239)**
Gly/Gly	2 (2.5)	1 (2.86)	1.182 (0.102-13.681)
Val/Gly or Gly/Gly	36 (45)	9 (25.71)	2.364 (0.984-5.680)
GADD45-IVS3+168			
TT	35 (43.75)	18 (51.43)	1
CT	40 (50)	13 (37.14)	1.582 (0.679-3.685)
CC	5 (6.25)	4 (11.43)	0.643 (0.153-2.692)
CT or CC	45 (56.25)	17 (48.57)	1.361 (0.614-3.019)
PTEN-IVS4+109			
del/del	37 (46.25)	12 (34.29)	1
del/ins	35 (43.75)	20 (57.14)	0.568 (0.242-1.331)
ins/ins	8 (10)	3 (8.57)	0.865 (0.197-3.792)
ins/ins or del/ins	43 (53.75)	23 (65.71)	0.606 (0.266-1.383)

Estrogen and Carcinogen Metabolism Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
ER-Ser10Ser			
TT	16 (20)	14 (40)	1
CT	40 (50)	13 (37.14)	**2.692 (1.039-6.975)**
CC	24 (30)	8 (22.86)	2.625 (0.896-7.688)
CT or CC	64 (80)	21 (60)	**2.667 (1.117-6.367)**
ER-Pro325Pro			
CC	46 (57.5)	19 (54.29)	1

CG	27 (33.75)	14 (40)	0.797 (0.345-1.842)
GG	7 (8.75)	2 (5.71)	1.445 (0.275-7.598)
CG or GG	34 (42.5)	16 (45.71)	0.878 (0.395-1.952)
CYP17-CACbox			
TT	27 (33.75)	17 (48.57)	1
CT	44 (55)	15 (42.86)	1.847 (0.794-4.294)
CC	9 (11.25)	3 (8.57)	1.889 (0.447-7.978)
CT or CC	53 (66.25)	18 (51.43)	1.854 (0.826-4.162)
COMT-Met158Val			
Met/Met	14 (17.5)	12 (34.29)	1
Met/Val	52 (65)	17 (48.57)	**2.622 (1.018-6.751)**
Val/Val	14 (17.5)	6 (17.14)	2 (0.586-6.833)
Met/Val or Val/Val	66 (82.5)	23 (65.71)	2.46 (0.995-6.083)
GSTM3-IVS6+20			
ins/ins	56 (70.89)	28 (80)	1
ins/del	21 (26.58)	6 (17.14)	1.75 (0.635-4.826)
del/del	2 (2.53)	1 (2.86)	1 (0.087-11.507)
del/del or ins/del	23 (29.11)	7 (20)	1.642 (0.629-4.289)
GSTP1-Ile105Val			
AA	39 (49.37)	13 (37.14)	1
AG	32 (40.51)	18 (51.43)	0.593 (0.253-1.391)
GG	8 (10.13)	4 (11.43)	0.667 (0.172-2.583)
AG or GG	40 (50.63)	22 (62.86)	0.606 (0.268-1.370)
DNA Repair and Other Cancer Related Genes			
Genotype	*Cases n (%)*	*Controls n (%)*	*OR (95% CI)*
XPD-Lys751Gln			
Lys/Lys	28 (35)	20 (57.14)	1
Lys/Gln	44 (55)	13 (37.14)	**2.418 (1.040-5.622)**
Gln/Gln	8 (10)	2 (5.71)	2.857 (0.547-14.912)
Gln/Gln or Lys/Gln	52 (65)	15 (42.86)	**2.476 (1.099-5.577)**
MTHFR-Ala222Val			
Ala/Ala	29 (36.25)	16 (45.71)	1
Ala/Val	39 (48.75)	15 (42.86)	1.434 (0.611-3.365)
Val/Val	12 (15)	4 (11.43)	1.655 (0.458-5.987)
Ala/Val or Val/Val	51 (63.75)	19 (54.29)	1.481 (0.661-3.317)
BARD1-Pro24Ser			
Pro/Pro	38 (47.5)	12 (34.29)	1
Pro/Ser	38 (47.5)	20 (57.14)	0.6 (0.258-1.397)
Ser/Ser	4 (5)	3 (8.57)	0.421 (0.082-2.152)
Pro/Ser or Ser/Ser	42 (52.5)	23 (65.71)	0.577 (0.253-1.315)
MMP1-(-1607) insG			
del/del	18 (22.5)	10 (28.57)	1
del/ins	39 (48.75)	16 (45.71)	1.354 (0.515-3.564)
ins/ins	23 (28.75)	9 (25.71)	1.42 (0.477-4.229)
ins/ins or del/ins	62 (77.5)	25 (71.43)	1.378 (0.559-3.395)

E. Immediate Future Task

We will continue to apply the statistical analysis to the SNP data. During this we will investigate the effect of gene-gene interaction in breast cancer risk. We will also investigate the risk associated by these SNPs in the context of gene-environment interactions focusing on relevant epidemiological risk factors for breast cancer risk.

F. Key Research Accomplishments

We have accomplished the tasks proposed in the Statement of Work by

- Establishment and validation of SNP genotyping methods
- Genotyping of 398 cases and 372 controls for 21 SNPs
- Validation of the quality of the genotyping data
- Initial statistical analysis to evaluate the breast cancer risk contributed by the SNPs
- Detection of associations between several SNPs and breast cancer risk (these findings are being interpreted at the current time)

- We have accumulated significant data from this study which has already initiated the application of novel statistical tools to understand the gene-gene interactions in breast cancer predisposition.

G. Reportable Outcomes

G1. Presentations

Venus Onay, Julia Knight, and Hilmi Ozcelik, "Microarray Technology to Study the Role of Candidate SNPs in Breast Cancer Risk" 3rd International Meeting on Single Nucleotide Polymorphism and Complex Genome Analysis, 8th-11th September 2000, Taos, New Mexico, USA.

Venus Onay, Julia Knight, and Hilmi Ozcelik, "Identifying the Role of SNPs in Breast Cancer Risk Using Microarray Technology." Oncogenomics Conference, 25-27 January 2001, Tucson, Arizona, USA.

Venus Onay, Julia Knight, and Hilmi Ozcelik, "Candidate SNP Analysis in the Study of Breast Cancer Risk Using SNParrays" 93rd Annual Meeting of AACR, April 6-10,2002, San Francisco, California, USA.

Venus Onay, Julia Knight, and Hilmi Ozcelik, "Candidate SNP Analysis in the Study of Breast Cancer Risk Using SNParrays" Controversies in the Etiology, Detection and Treatment of Breast Cancer:2002, June 13-14,2002, Toronto, Ontario, Canada.

Venus Onay, Julia Knight, Sean Wells, and Hilmi Ozcelik, "Candidate SNP Analysis in the Study of Breast Cancer Risk Using SNParrays" The 4th Era of Hope Meeting for the Department of Defense Breast Cancer Research Program, September 25-28, 2002, Orlando, Florida, USA.

U. Venus Onay, Julia Knight, Sean Wells, Hong Li, and Hilmi Ozcelik, "Investigating the Role of 24 Variants from Major Cancer Related Pathways in Breast Cancer," Cancer Family Registries of Breast and Colon Cancer, Scientific Meeting, January 15-17, 2003, Waikoloa, Hawaii.

U. Venus Onay, Julia Knight, Sean Wells, Hong Li, and Hilmi Ozcelik, "Investigating the Role of 24 Variants from Major Cancer Related Pathways in Breast Cancer," AACR International Conference on Molecular and Genetic Epidemiology of Cancer; January 18-23, 2003, Waikoloa, Hawaii.

G2. Publications:

Onay UV, Knight JA, Wells S, Hong L, Andrulis IL, Briollais L, Ozcelik H., "Genetic Variants of Cell Cycle Genes and Breast Cancer Risk" (in preperation).

Onay UV, Figueiredo J, Knight JA, Wells S, Hong L, Andrulis IL, Briollais L, Ozcelik H., "A DNA repair SNP, XPD 751, and Breast Cancer Risk" (in preperation).

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